Chemical Research in Toxicology

Rapid Report

Cite This: Chem. Res. Toxicol. 2018, 31, 431−434

pubs.acs.org/crt

Free-Base Nicotine Determination in Electronic Cigarette Liquids by ^{1}H NMR Spectroscopy

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Supporting Information

ABSTRACT: E-liquids usually contain significant nicotine, which will exist primarily in two forms, monoprotonated and free-base, the proportions of which are alterable through the effective pH of the medium. The fraction of nicotine in the free-base form is α_{fb}, with $0 \leq \alpha_{fb} \leq 1$. When dosed via aerosol, the two nicotine forms have different mechanisms and kinetics of delivery, as well as differing implications for harshness of the inhaled aerosol, so α_{fb} is relevant regarding abuse liability. Previous attempts to determine α_{fb} in electronic cigarette liquids and vapor have been flawed. We employed the exchange-averaged ^{1}H NMR chemical shifts of nicotine to determine α_{fb} in samples of e-liquids. This method is rapid and direct and can also be used with collected aerosol material. The e-liquids tested were found to have $0.03 \leq \alpha_{fb} \leq 0.84$. The α_{fb} values in collected aerosol liquid samples were highly correlated with those for the parent e-liquids. E-liquids designed to combine high total nicotine level (addictive delivery) with low α_{fb} (for ease of inhalation) are likely to be particularly problematic for public health.

In the United States during 2016, electronic cigarettes (e-cigarettes) were used regularly by ~8 million adults.[1,2] For high school students, CDC surveys estimate e-cigarette use in 2013, 2014, 2015, and 2016 to have been 5, 13, 16, and 11%, respectively, and for conventional cigarettes 13, 9, 9, and 8%, respectively.[2,3] Often argued[4] though not proven to be safer than conventional cigarettes,[5,6] e-cigarettes are not, in any case, risk free. And, many e-cigarette liquids (e-liquids) contain substantial nicotine, which is addictive and can be toxic.

Nicotine has three forms: free-base (Nic, aka unprotonated), monoprotonated ($NicH^+$), and diprotonated ($NicH_2^{2+}$). The protonation state of nicotine can be altered by changing the acid/base conditions in the medium.[7,8] In water at 25 °C, pK_1 (for $NicH_2^{2+}$) and pK_2 (for $NicH^+$) are 3.10 and 8.01, respectively.[9] Tobacco smoke aerosols are believed to contain primarily the Nic and $NicH^+$ forms (Figure 1) because conditions in the aerosol particulate material (PM) are not considered to be sufficiently acidic to generate significant $NicH_2^{2+}$.[7,8]

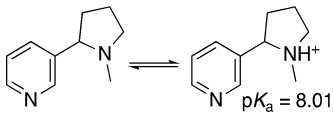

Figure 1. Distribution of nicotine in vape and tobacco aerosols primarily involves two forms: (left) Nic (free-base) which has volatility; and (right) $NicH^+$ (monoprotonated) which is nonvolatile. The fraction of the free-base for (α_{fb}) depends on the acid/base conditions. In water at 25 °C, pK_a = 8.01.

The fraction of nicotine in the free-base form is α_{fb}, with $0 \leq \alpha_{fb} \leq 1$:

$$\alpha_{fb} \equiv \frac{[Nic]}{[Nic] + [NicH^+]} \quad (1)$$

where $NicH_2^{2+}$ is neglected. The α_{fb} can affect the kinetics and location of nicotine uptake from an inhaled aerosol because the free-base form is volatile: it can deposit from an inhaled tobacco smoke (or vape) aerosol from the gas phase *and* by particle deposition, whereas only particle deposition is operative for protonated nicotine.[10] It has been argued that these considerations make it likely that α_{fb} affects nicotine addiction potential.[11,12] In addition, high α_{fb} values have long been connected with tobacco smoke harshness upon inhalation.[13]

In water, neglecting $NicH_2^{2+}$

$$\alpha_{fb} = \frac{1}{1 + 10^{-pH}/K_a} \quad (2)$$

where K_a is the acidity constant for $NicH^+$ in water (K_2 as given above). Other than nicotine level, commercial labels on e-liquid products currently provide little compositional information, and these labels certainly do not indicate α_{fb} values.

Historically, methods for determination of α_{fb} in tobacco smoke PM have been flawed.[10] One method introduced a significant amount of water for subsequent measurement of the pH of the aqueous phase,[14] and a second introduced water and

Received: April 10, 2018
Published: May 18, 2018

DOI: 10.1021/acs.chemrestox.8b00097
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an organic solvent (e.g., chloroform) for what was intended to be a selective extraction of the neutral free-base form.[15] Given the disrupting effects of added liquids, neither method can give good results. Pankow et al.[16] describe a successful method for α_{fb} determination in tobacco smoke PM that uses equilibration with a gas volume as a means to detect volatile nicotine, which is taken to be proportional to α_{fb}. In addition, direct measurement by ^{1}H NMR spectroscopy of α_{fb} is possible for tobacco smoke PM[17] and for PM from the now-defunct Eclipse product[7] which gave aerosols compositionally similar to those from e-liquids. (Others attempted using NMR, but added a solvent that will perturb α_{fb}.[18]) Our work reported here describes the development of ^{1}H NMR spectroscopy for measurement of α_{fb} in e-liquids and their aerosols. The materials and methods are provided in the Supporting Information.

For each sample, nicotine ^{1}H chemical shifts (δ) were measured for different protons on the nicotine molecule (H_a through H_e). The assignments are in accordance with those previously made[17] and verified by the J-coupling patterns and integrations. The δ of H_e was subtracted from H_a through H_d to obtain the difference, $\Delta\delta$, as in eq 3, noting that $\Delta\delta$ depends on its position in the molecule, that is, some of the protons shift more than others.

$$\Delta\delta = [\delta H_{\text{aromatic proton}}(\text{i.e., } H_a \text{ through } H_d)] - [\delta H_e] \quad (3)$$

Nicotine standards (24 mg nicotine / mL in PG/GL mixtures; see Supporting Information) were then used to calculate $\Delta\delta$ for the monoprotonated and free-base states of nicotine after assessment with a variety of acids and concentrations thereof. In practice, we used only the aromatic protons H_a and H_b to avoid steric or direct charge contributions that may affect the chemical shifts of H_c and H_d; these protons being proximal to the nicotine pyrrolidine ring. Commercial e-liquid samples were then evaluated by the use of eq 4, with the resonances indicated in Figure 2:[17]

$$\alpha_{fb} = \frac{[(\Delta\delta_{\text{commercial sample}}) - (\Delta\delta_{\text{monoprotonated sample}})]}{[(\Delta\delta_{\text{free-base standard}}) - (\Delta\delta_{\text{monoprotonated standard}})]} \quad (4)$$

Thus, for "Taurus" (using the H_a and H_e chemical shifts):

$$\alpha_{fb} = \frac{[(6.120\,\text{ppm}) - (5.942\,\text{ppm})]}{[(6.331\,\text{ppm}) - (5.942\,\text{ppm})]} = 0.46 \quad (5)$$

Free-base fractions (α_{fb}) for a selection of commercial e-liquids were also calculated; the results are shown in Figure 3, with α_{fb} ranging from 0.03 to 0.84.

The accuracy of the method was verified by adding acid and base, respectively, to "Zen" flavored e-liquid aliquots. The resulting free-base and protonated direct chemical shift values were used to calculate α_{fb} = 0.83 ± 0.00 (range), which was statistically equal to the overall-calibration derived value of 0.84 ± 0.01 (range), using eq 4 as before.

As an initial examination of how vaporization may affect α_{fb}, e-liquids with high and low α_{fb} values were vaporized, and the PM collected and analyzed. The "Zen" e-liquid, which had the highest free-base content of the e-liquids tested, was found to have a post-vaporization α_{fb} of 0.80 ± 0.01 (range), which is similar to the unvaporized value of 0.84 ± 0.01. "Maui" (24 mg/mL) was determined to have a post-vaporization α_{fb} of 0.78 ± 0.01 (range), which is comparable to the unvaporized α_{fb}, which was 0.80 ± 0.00. The JUUL "crème brulee" flavored e-liquid was found to have a post-vaporization α_{fb} of 0.05 ± 0.03 (range), also comparable to its unvaporized value of 0.07 ± 0.02. JUUL e-liquids are advertised to contain benzoic acid, which we verified by NMR as being present primarily in its ionic, benzoate form.

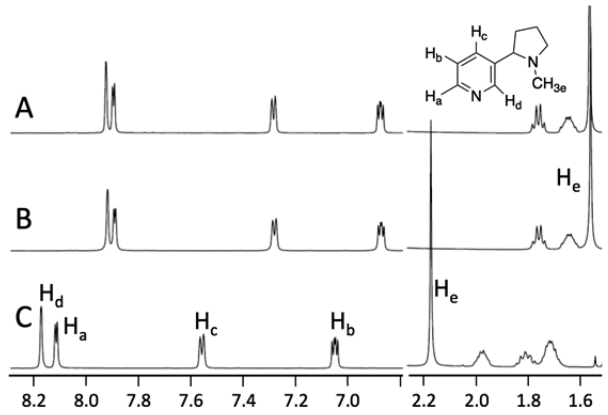

Figure 2. ^{1}H NMR spectra showing the chemical shift changes for nicotine in a propylene glycol + glycerol (PG + GL) stock mixture with the addition of acid and base, independently. (A) 1 × *t*-butylamine added (relative to moles nicotine). (B) PG + GL e-liquid stock (no acid or base additives). (C) 5 × acetic acid added. Stock mixture contained 54 PG:46 GL (by moles) and 24 mg/mL nicotine. Samples were prepared by isolating the e-liquid sample in an inner concentric NMR tube, with DMSO-d_6 lock solvent in the outer tube, at 40 °C.

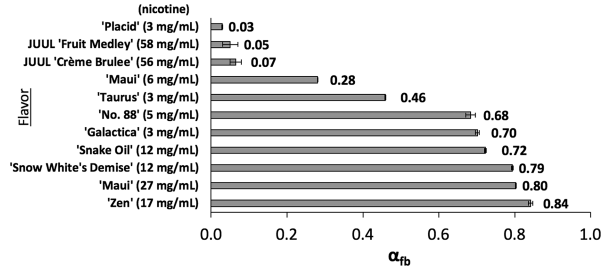

Figure 3. Free-base nicotine fraction (α_{fb}) in commercial e-liquids as an average using aromatic protons H_a and H_b. The ranges between free base values are indicated. Nicotine amounts as indicated to the right of each name were determined by NMR integrations, relative to the PG and GL resonances.

The NMR method presented here may be compared with contemporary analogs for e-liquids of the two historical methods for α_{fb} in tobacco smoke PM. First, Stepanov and Fujioka,[19] Lisko et al.,[20] and El-Hellani et al.[8] all describe diluting an aliquot of e-liquid with water, measuring the pH, and then calculating α_{fb} by eq 2. The result is that the values obtained suffer from both medium effects (water is different from an e-liquid) and dilution, though the pH values may, nevertheless, provide some useful relative indications of the overall acid/base balances in different e-liquids. However, that can be compromised if air-related CO_2 is present in the added water and affects the measured pH values. This problem is likely evidenced in the data of Lisko et al.[20] (see Supporting Information). Second, El-Hellani et al.[8] describe making 6 mL aqueous solutions of e-liquids, extracting with 6 mL toluene, and then determining nicotine in the toluene solvent extract as a measure of the nicotine percentage in the water. This approach suffers from the same dilution, medium, possible CO_2 incursion effects discussed above and introduces uncertainties

DOI: 10.1021/acs.chemrestox.8b00097
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regarding the extent to which the toluene extraction step affects the position of the $NicH^+ \leftrightarrows Nic + H^+$ equilibrium in the aqueous dilution.

In order to confirm the above concern directly, the JUUL "crème brulee" e-liquid was diluted into D_2O to determine if α_{fb} was affected by dilution into this deuterium analog of water. The dilution (5:1, by volume) was found to result in fully monoprotonated nicotine.

Although we used a 600 MHz NMR system for this work, it is possible that these methods could be adapted for lower field NMR, and even benchtop instruments. This is a rapid and easy way to measure α_{fb} in e-liquids accurately and may be of interest to those concerned with addiction and regulation.

In summary, α_{fb} of e-liquids can be determined directly by ^1H NMR using protonation-dependent chemical shifts for nicotine. In a small number of tests, α_{fb} values were found to be largely unaffected by the vaping process. Of the products tested, only the JUUL liquids were found to combine high nicotine levels with low α_{fb} values. Pharmacokinetic uptake rates for nicotine may vary among the products, and certainly tobacco company documents (e.g., Chen)[13] suggest that products with high nicotine levels but low α_{fb} such as JUUL will yield vape aerosols of much reduced harshness as compared to products with even only moderate nicotine levels but $\alpha_{fb} \approx 1$. This may well contribute to the current use prevalence[21] of JUUL products among youth.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.chemrestox.8b00097.

Materials and Methods section and a discussion of "pH of water dilutions of electronic cigarette fluids" (PDF)

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Funding

This work was supported by the U.S. National Institutes of Health, grant R01ES025257. Research reported was supported by the NIEHS and FDA Center for Tobacco Products (CTP). The content is solely the responsibility of the authors and does not necessarily represent the views of the NIH or the FDA.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Thank you to Kevin McWhirter for the preparation of some of the sample mixtures.

■ ABBREVIATIONS

PG, propylene glycol; GL, glycerol; e-cigarettes, electronic cigarettes

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